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This meeting gave an excellent overview of the recent developments in gene therapy. Much research effort has focused on the improvement and de novo construction of gene vectors, on characterizing their mechanisms of action and on their interactions with, and in, living organisms. The continual improvements in understanding disease at a molecular level and the progress in cell biology, immunology and related fields have opened the way for novel gene therapy approaches. The gene therapeutic strategy has proven to be feasible and efficient in numerous preclinical (animal) models of a variety of diseases. On the other hand, applying this experience to humans has turned out to be difficult. Currently, the field is rapidly moving into clinical applications. No major limiting side effects have been observed in patients in the phase I and later stage trials presented. Nonetheless, years of preclinical and clinical research will be required before gene therapy can be considered a reliable and efficient therapeutic approach with broad applicability.

Introduction

This meeting, organized by B Gänsbacher and his co-workers (Technical University of Munich, Germany), succeeded in covering the relevant current topics in the field, comprising the latest developments in viral and non-viral vector construction, preclinical assessment and optimization and highlights of recent clinical studies. The meeting featured 56 invited speakers, predominantly from Europe and gave ample time for 19 oral presentations selected from more than 250 posters on display. The program also included satellite meetings organized by Euregenethy on regulatory issues and by ESACT on industrial aspects of gene therapy. Approximately 600 participants from all over the world attended the lectures, and more than 40 journalists participated in the meeting press conference.

Vector development

Viral vectors

The common theme of most talks was the modification of the viral envelope to enhance infectivity/transduction frequency or to change host tropism. In this context, L Naldini (University of Turin, Italy) reported on a hybrid lentiviral vector, which contained the *gag*, *pol* and *rev* genes of HIV-1 but not the envelope of VSV. This combination led to extension of the viral tropism to several rodent tissues *in vivo*- as well as to *ex vivo*-isolated human hematopoietic stem cells. Generation of inducible, packaging cell lines further led to high yields of virus, and hence, to further enhancement of transduction efficiency, broadening the host range even further.

Another approach to modifying the envelope takes advantage of the well-characterized C-type retrovirus mode of targeting. S Russell *et al* (Mayo Foundation, Rochester, MN, USA) have extended the envelopes of MLV by sequences of Factor Xa, matrix metalloproteinase (MMP)-cleavable or non-cleavable EGF domains. Infectivity of a broad range of cells expressing tissue factor or EGFR, respectively, was achieved. Also, a new and efficient method of virus concentration was developed.

For specific targeting of non-dividing human hematopoietic cells, another study by K Cichutek *et al* (Paul Ehrlich Institut, Langen, Germany) employed human or feline lentiviral-modified envelopes of viruses, such as MLV or SNV. In addition, scFv was incorporated into the envelopes to target lymphoid cells. Incorporation of the nuclear localization signal into the matrix protein of SNV led to more efficient transduction of growth-arrested cells. To circumvent the requirement of activation of CD4+ lymphocytes for their efficient infection by lentiviral vectors, FL Cosset (INSERM, Lyon, France) reported on the development of chimeric envelope proteins, which include polypeptides capable of interacting with and stimulating receptors, leading to transient activation of the target cell. M Collins *et al* (Windeyer Institute, London, UK) have targeted MLV-based vectors on human melanoma tumors by the fusion of a melanoma-specific antibody (against the HMW-MAA antigen) to the envelope of MLV. The scFv is susceptible to cleavage by MMP, which, after attachment of the virus to the melanoma, leads to infection of the targeted cell.

M Hallek *et al* (University of Munich, Germany) improved AAV-based vectors by optimization of packaging and purification, leading to high virus titers. In addition, a non-viral ligand was introduced into the viral capsid, which improved the host range of target cells. K Joos *et al* (Genethon III, Evry, France) investigated immune activation after AAV and Ad gene transfer to transgenic animals expressing the influenza virus, hemagglutinin-specific T-cell receptor on 10 % of their CD4+ T-cells. The results clearly showed that an immune response was elicited for both vector and transgene in adenoviral delivery. In the case of AAV, the only immune response observed was against the transgene and depended on its cellular localization.

S Kochanek (University of Cologne, Germany) presented data comparing first generation Ad vectors and high-capacity Ad (HC-Ad) in transferring the α_1 antitrypsin gene to the liver. Stable, relatively high-level, long-term expression in immunocompetent mice was achieved with HC vectors, although liver toxicity due to viral particles was observed. Details of new, more efficient packaging cell lines for helper removal and an E1A/B-expressing cell line

derived from amniocytes, which do not produce RCA, were presented. E Vigne (Rhône-Poulenc Rorer SA, Vitry-sur-Seine, France) presented data on different strategies for modification of Ad tropism. Modifications of the fiber knob, but not in the viral hexon, resulted in stable and productive virions when the uPAR recognition domain was introduced and was able to infect cells with little or no CAR receptor on their surfaces. This ability was efficient *in vitro* and less efficient *in vivo*. Other approaches involve fiber knob mutants with decreased affinity for CAR and short shaft fibers.

Lentiviral vectors have been used by D Trono and *et al* (Centre Médical Universitaire de Genève, Switzerland), to transfer growth-promoting, anti-senescence and anti-apoptotic genes into human primary cells. The vectors are constructed in such a way that the integrated gene can be excised with cre recombinase, leading to a reversibly immortalized phenotype ('lentimortalization'). C Buchholz (Paul Ehrlich Institute, Langen, Germany) demonstrated the utility of retroviral display libraries in selecting virus with desired targeting capacity (such as targeting of T-cells) and also in identifying potential cleavage sites of cellular proteinases. A Hüser and co-workers (HepaVec AG, Berlin, Germany) constructed baculovirus vectors, which are targeted to specific cell types, by genetically modifying the envelope protein, gp64. This concept was illustrated by targeting the EGF receptor of cells that are otherwise not susceptible to baculovirus infection. L Weiß (MondoGen GmbH, Martinsried, Gemany) talked about the use of hepatitis B virus (HBV) as a liver-specific vector. He and his co-workers succeeded in constructing non-virulent, recombinant HBV particles that carry foreign genes. A Richters (Heinrich-Pette-Institute, Hamburg, Germany) reported on the modification of retroviral vectors to improve their expression in hematopoietic and lymphoid cells. Deletion of putative Sp1 binding sites in the enhancer of the murine leukemia virus SFFVp and modification of the 5-untranslated leader led to increased transgene expression in hematopoietic and lymphoid cells.

Non-viral vectors

The investigation of techniques to physically and biologically stabilize polycation-DNA complexes (polyplexes) during the delivery phase, by grafting PEG layers, is being undertaken by J-P Behr (University of Strasbourg, France), C Plank (Technical University of Munich, Germany) and E Wagner (Boehringer Ingelheim Austria GmbH, Vienna, Austria). The grafting is done on pre-assembled vector particles, either by covalent linkage or by electrostatic interaction of negatively-charged protective copolymers ('PROCOPs') with the particle surface. With appropriate vectors, transfection of various organs with protected polyethyleneimine (PEI)-DNA complexes is feasible upon iv or intratracheal application (liver, lung, spleen, heart). E Wagner reported on gene transfer into distal tumors upon iv application of various vector formulations. In particular, the PEG formulations displayed improved performance.

D Scherman (Rhône-Poulenc Rorer SA, Vitry-sur-Seine, France) reported on achievements with electroporation-assisted transfection of naked DNA into skeletal muscle. This application modality provides impressively increased transfection efficiency and greatly reduced transgene expression variability when compared to simple im injection. Furthermore, physiologically relevant levels of therapeutic products, such as FGF1, are expressed long-term (> 9 months) in animal models (mouse, rat, rabbit, monkey). M Blaese (Kimeragen Inc, Newtown, PA, USA) reported on the optimization of so-called chimeraplast technology. The current optimized chimeraplasts are DNA-RNA hybrid 68-mer oligonucleotides having a 25 to 30 bp homology to the target sequence, with a single mismatch in the DNA moiety, while the RNA moiety seems to be required for sequence targeting. These molecules can correct single base mutations involving the cellular repair machinery in bacteria, fungi, plants, fish, rodents and humans. These molecules are precise (do not alter non-target regions) and are delivered with PEI *in vivo*. Up to 40% target conversion in the liver of rats has been reported. As only few investigators were able to reproduce the results, the technique is highly controversial. M Blaese pointed out that the chimeraplast needs to be of highest purity in order to be functional, which is difficult to achieve with current synthetic methods.

Among the numerous posters presented in the non-viral vector field, particular attention was given to novel peptidic delivery agents. These included a peptide nucleic acid (PNA)-nuclear localization signal construct presented by L Brandén (Karolinska Institute, Stockholm, Sweden), a fusion protein comprising a GAL4 binding domain and a novel cell-permeable peptide leading to high-efficiency gene transfer *in vitro* presented by S Oess and E Hildt (Technical University of Munich, Germany), a nuclear localization peptide construct ('GenePort') presented by W Ritter (University of Munich, Germany), and a peptide ligand for gene transfer into lung epithelial cells selected by phage display presented by P Jost (Imperial College of Science, Technology & Medicine, London, UK).

Cardiovascular disorders

S Ylä-Herttuala (University of Kuopio, Finland) outlined current approaches towards gene therapy for restenosis after angioplasty/stenting of peripheral and coronary arteries. He showed cell culture data and a phase I evaluation of both plasmid and adenoviral gene transfer of vascular endothelial growth factor (VEGF) into the vessel wall, using a special device which allows full perfusion of the distal vessel. His data on 15 patients show that gene transfer is feasible. No major adverse reactions were observed; data on clinical outcome were not presented. C Kalka (Tufts University School of Medicine, Boston, MA, USA) reported on 3 years experience of inducing angiogenesis in angiopathic patients, with plasmid-based VEGF gene transfer. Positive results were reported, however, no control group was included so that the results of an ongoing, randomized, controlled study, will be of great interest. Additionally, the group's efforts to induce neovascularization by using endothelial stem cells merit attention.

Complete regression of atherosclerosis after adenoviral gene transfer of human apolipoproteins E and A-I in a nude mouse model with an *apoE* gene knockout, was demonstrated. D Branellec (RPR Genceil, Vitry-sur-Seine, France) demonstrated complete regression of atherosclerosis after adenoviral gene transfer of human apolipoprotein

(apoE) and A-1 in a nude mouse model with an *apoE* knockout gene. The group is now developing vectors for human application. D Branellec also reported on the *FGF1* gene contained in a plasmid and grown in a particular strain of *E coli* that does not require antibiotic selection. This construct was electrotransferred into muscle and revascularization was demonstrated in rabbits. A clinical trial in humans began in 1999. S Kingsman (Oxford Biomedica Ltd, Oxford, UK) showed work on hypoxia-inducible promoters used in various viral vector systems. The strength of such promoters under hypoxic conditions is comparable to that of the CMV promoter. In an effort to enhance gene transfer to underperfused and hypoxic solid tumors, *ex vivo* viral gene transfer to macrophages from peripheral blood was performed. These macrophages were used for active invasion and gene expression in otherwise inaccessible tumor spheroids, and in mice bearing human tumor xenograft.

Cancer

R Bolhuis (Daniel den Hoed Cancer Center, Rotterdam, The Netherlands) reported an approach for renal cell carcinoma, using single chain antibodies (scFv) consisting of one or two chains of the TCR, with specificity for a tumor-associated antigen (TAA). Peripheral blood lymphocytes are taken from a patient, transduced *ex vivo*, activated and subsequently tested for their specificity of lysis of TAA-positive or -negative cells. Specific lysis of TAA-expressing tumor cells was observed. L Eisenbach (Weizmann Institute of Science, Rehovot, Israel) presented their transgenic mouse system for expression of single-chain HLA-A2-CTL (HDD). This system is useful for identifying new TAA peptides of, eg, breast, prostate, colon or transitional cell carcinoma. Examples of peptides identified and attempts to specifically modify these peptides to achieve better lysis by CTL were presented. Z Eshhar (Weizmann Institute of Science, Rehovot, Israel) presented the 'T-Body' approach for expanding effector lymphocytes and redirecting them to specific targets. In this approach variable regions of mAb are combined with effector domains. Different regions of the TCR or FcR were used. Improvements were achieved by using hinges to connect scFv. In a prostate CA model (SCID), lymphocyte activation and redirection to the tumor cells was demonstrated, using patient tumor cells and patient lymphocytes retrovirally-transduced with the chimeric receptor genes.

M Ford (Glaxo Wellcome R&D, Stevenage, UK) discussed gene-directed enzyme therapy. He presented different suicide genes, emphasizing the nitroreductase (*NTR*) gene. NTR, expressed upon transduction with various viral vectors, was found to kill cells in the presence of the prodrug, CB-1954 (Glaxo Wellcome plc), *in vitro* as well as *in vivo*, exhibiting a bystander effect in cell-mixing experiments. The CEA promoter was used in retroviruses to direct expression of CD for targeting hepatic metastases of colorectal cancer. A reduction in tumor size was observed after treatment with 5-FC. D Klatzmann (Hôpital de la Pitié Salpêtriere, Paris, France) presented data on the *in situ* generation of retroviral vectors in cancer cells. This is achieved by multiple infection of cells with different vectors carrying the required genetic information for the assembly of a retroviral vector carrying the therapeutic gene. The retroviral particles produced *in situ* are able to infect neighboring tumor cells. For safety reasons, it was shown that AZT could block RV replication. T Kipps (University of California San Diego, La Jolla, CA, USA) reported on a strategy for inducing an immune response against B-cell tumor cells (chronic lymphocytic leukemia). In a dose-ranging phase I trial, B-cells were removed by leukapheresis, transduced with an adenoviral vector carrying the genetic information for the CD40 ligand (CD154), and re-injected into the patient in a single iv infusion. Nearly all patients showed reductions in blood leukemia cell counts and an increase in absolute T-cell numbers, while no dose-limiting toxicity was observed.

Data obtained for the replicating, attenuated Ad, ONYX-015 (ONYX Pharmaceuticals Inc), in phase I/II clinical studies with head and neck cancer, were presented by D Kirn (ONYX Pharmaceuticals Inc, Richmond, CA, USA). Data for intratumoral injection of ONYX-015 in combination with systemic cisplatin chemotherapy were shown, including results of a phase I/II clinical trial with head and neck cancer with repeated injection at the interface between tumor and normal surrounding tissue. Flu-like side effects and local pain were observed after repeated injection of 1×10^{10} pfu. Replication was seen in five of seven patients at days 5 to 8 of biopsies but, at day 22, no replication was observed in any patients. Restriction of Ad to tumor versus normal cells was shown in one example. Dose escalation trials with hepatic carcinoma and non-small cell lung cancer (NSCLC) were described in which the administration of up to 1×10^{11} pfu (hepatic artery) and 1×10^{12} pfu (NSCLC) proceeded without severe side effects. M Mehtali (Transgene SA, Strasbourg, France) reported on combination therapies for the treatment of local and metastatic tumors. In a large study in animals, adenoviral vectors expressing either cytokines or suicide genes alone or in combination, were compared. For particularly aggressive tumors, the introduction of a novel suicide gene, comprising a fusion between the yeast *FCY1* and *FUR1* genes, increased survival dramatically when used in combination with an IL2-expressing adenoviral vector.

C Traversari (Istituto Scientifico H San Raffaele, Milan, Italy) reported that dendritic cells are able to acquire surface antigens, such as vector-encoded LNGFR or HLA molecules, from cells with which they are co-cultivated. This acquisition requires cell to cell contact and allows recognition of the DC cells modified in this way by allospecific T-lymphocytes. W Weis (Georg-Speyer-Haus, Frankfurt, Germany) reported on the construction of a tumor-targeted, non-viral vector exploiting the targeting capacity on scFv antibody fragments to tumor associated antigens. In a second approach, a gene for a bispecific, co-stimulatory molecule was transfected with such a vector and expressed. The gene product (a fusion protein of a tumor-specific scFv and the B7 protein) was shown to localize to the tumor cell surface thus providing a co-stimulatory signal for T-cell activation. Application of this method in a mouse tumor model facilitated tumor rejection.

A dose-ranging, phase I clinical trial of first-generation adenoviral vectors expressing p53, for the treatment of advanced bladder cancer, was also reported. This trial utilized intratumoral injection or washing of the bladder for

1 h. Interestingly, transduction efficiency was enhanced by the addition of ethanol. C Klein (Harvard Medical School, Boston, MA, USA), reported on the superiority of dendritic cells, engineered to express specific tumor antigens, as an antitumor vaccine over tumor cells engineered to express cytokines, such as IL-2. Moreover, the additional expression of a chimeric E/L selectin allowed these dendritic cells to interact with high endothelial venules. B Salmons (Bavarian Nordic, Martinsried, Germany) reported on the use of cells overexpressing cytochrome p450, as a therapeutic gene, encapsulated in polymeric cellulose phosphate, for the treatment of pancreatic cancer. The encapsulation of the producer cells allowed local expression and thereby localized cytotoxicity of cytochome p450 and prodrug treatment in animals. A phase I study indicated that this approach limits tumor progression and increases overall survival time. A multicenter phase II trial is in preparation.

Hematopoietic cell disorders

C Baum (Heinrich-Pette-Institut, University of Hamburg, Germany) described the use of retroviral vectors for the transduction of CD34+ cells with the multi-drug resistance (MDR) gene, for hematopoietic cell protection during chemotherapy. Modification of T-cells is also to be exploited for allogeneic hematopoietic cell transplantation. A Fischer (INSERM U429, Paris, France) reported on retroviral delivery of the γc subunit of IL-2, -4, -7, -9 and -15, for the treatment of severe, combined immunodefficiency X1. After correction of the disease in the mouse animal model, a clinical trial was started. Cytokine-activated CD34+ cells from patients were transduced *ex vivo* with the γc vector and subsequently re-injected into the patients. After 6 months, the two treated patients are at home, off medication and have normal γc(+) T-cell counts which were functionally active. S Karlsson (Lund University, Sweden) described the optimization of a VSV-G pseudotyped lentiviral vector for transducing human CD34+ cells. Several promoters were compared, with the EF1α promoter giving the highest expression. The treated cells were able to graft into NOD/SCID mice and could be transplanted from one mouse to another without loss of transgene expression.

C Kalle (University of Freiburg, Germany) described a ligase-mediated PCR method of following the fate of hematopoietic stem cells after autologous transplantation in a preclinical large animal model and in a clinical retroviral marking trial. With this method and direct genomic sequencing, hematopoietic lineage could be identified and clonal activity could be followed by conventional PCR at 180 days after transplantation into Rhesus monkeys. Gene transfer was observed in up to 1% of human hematopoietic repopulating cells. G Veres (Systemix Inc, Palo Alto, CA, USA) presented data on the use of pseudotyped HIV- and MLV-based vectors in transducing non-stimulated CD34+ cells. The HIV-based vectors, in contrast to the MLV-based vectors, were able to transduce > 30% of non-stimulated human CD34+ cells, which were able to repopulate human fetal thymic grafts in SCID-hu Thy/Liv mice.

Neuromuscular disorders

It was disclosed that P Aebischer and co-workers (Lausanne University Medical School, Switzerland) are using third-generation lentiviral vectors in order to express therapeutic proteins, such as GDNF and CNTF, for gene therapy of Parkinson's disease. In rodents and primates, sustained gene expression is found in a high percentage of the cells of the striatum or the substantia nigra, where GDNF induces the spreading of dopaminergic neurons. In a second approach, cells transduced *ex vivo* are encapsulated in a semi-permeable, hollow-fiber device that can be implanted in an animal or patient. Such devices can produce microgram quantities of therapeutic proteins over extended periods of time. A clinical trial with cells expressing GDNF is pending.

Duchenne's muscular dystrophy (DMD)

Data on the correction of the hereditary defect in the mdx transgenic mouse model were presented by E Burton (University of Oxford, UK). This was achieved with the help of adenoviral vectors capable of transducing the gene for utrophin, which may be able to replace dystrophin in DMD. In addition, this research group is screening small compound libraries in order to find inducers of the transcription of endogenous utrophin from its natural promoters in subsynaptic regions. G Dickson and co-workers (Royal Holloway College, University of London, UK) are investigating gene correction strategies for DMD. Based on the naturally occurring phenotype conversion caused by exon-skipping in the mdx mouse and humans, the group screened arrays of oligonucleotides to induce forced exon-skipping. With an oligo specific for the 5'-splice site of exon 23, fusion is forced to a downstream exon, which leads to functional dystrophin both *in vitro* and *in vivo* (mdx mouse). In another approach, the group is using chimeraplasts from Kimeragen Inc for gene correction with some success. H Lochmüller (Genzentrum, University of Munich, Germany) reported on transfer of the dystrophin gene using adenoviral vectors in combination with a cocktail of immunosuppressants such as FK-506 (Fujisawa Pharmaceutical Co Ltd) and CTLA4-Ig (RepliGen Corp). As immunological problems with adenoviral vectors are obvious, the group is trying to construct minidystrophin genes (6.3 kb) that fit into AAV vectors. A Pavirani presented Transgene's preclinical efforts in the DMD field with naked DNA, Ad and lentiviral gene delivery. The company is currently initializing clinical study (naked DNA delivery), the major question being whether there will be an immune response against the newly-expressed dystrophin gene. An alternative to classic gene delivery was presented by R Mulligan (Children's Hospital, Boston, MA, USA). Dystrophin has been delivered successfully to diseased muscle, using iv injection of engineered bone marrow stem cells. At 8 weeks post-transplantation, dystrophin-expressing donor cells were not only found in the bone marrow of the recipient, but also as differentiated cells in muscle which stained positive for dystrophin, as detected by FISH. Also, the differentiation of transplanted bone marrow cells to endothelial cells within 3 months post-transplantation was observed. Apart from many open questions, this is potentially a new and powerful means of treating a multitude of diseases.

Genetic and other organ diseases

Cystic fibrosis

E Alton (National Heart & Lung Institute, London, UK) and his group has applied formulations of lipid 67 from Genzyme Corp for gene transfer of CFTR. This lipid vector,

which has proven highly effective in previous, comprehensive screening studies, is applied by nebulization. Small but significant improvements in chloride transport in the lungs of CF patients were briefly observed. In this recently completed, phase 1 trial, mild flu-like symptoms were observed in the test patients but not in the placebo-treated patients.

Hunter syndrome

Adenoviral vectors for the iduronate-2-sulfatase (IDS) gene, which were administered into the knee joints of healthy rabbits have been developed by M Scarpa and co-workers (University of Padova, Italy). In order to evaluate the potentially limiting effect of antibodies against the vector, animals were pre-immunized with wild-type Ad. Expression of IDS was achieved with or without pre-immunization. No dissemination of the vector to other organs was observed. In a second approach, stably-transduced C2C12 cells are encapsulated into polyalginate capsules for implantation into recipients.

Chronic granulomatous disease

Work on the gene therapy of chronic granulomatous disease (CGD), which leads to impaired antimicrobial activity in afflicted patients was presented M Grez (Georg-Speyer-Haus, Frankfurt, Germany). The group has developed a bicistronic retroviral vector for the transduction and subsequent selection of CD34+ cells with the X-CGD gene ($gp91^{phox}$), which are then differentiated with the help of G-CSF and SCF. The performance of the strategy is assessed in a murine model of X-CGD, which demonstrates that, with an optimized protocol, up to 77% of peripheral blood neutrophils are positive for superoxide production, 7 months after transplantation of transduced cells.

Hemophilia

H Schneider and co-workers (Royal Free Hospital and University College Medical School, London, UK) are developing strategies for *in utero* gene delivery. The feasibility of this approach was demonstrated with ultrasound-guided percutaneous Ad delivery into the umbilical vein and subsequent expression of the transduced β-Gal or human factor IX gene in various tissues of fetuses and newborn lambs. Potential germ-line transmission still needs to be convincingly excluded.

A report by J Glorioso (University of Pittsburgh, PA, USA) on the use of HSV-based vectors for infecting peripheral neurons was presented. Using the latency promoter, LAP2, they directed the expression of nerve growth factor (NGF) for the treatment of bladder dysfunction in a rat diabetic model. After intra-articular delivery in rabbits, expression above baseline was stable over several months following administration. The maintenance of the viral genome in cells other than neurons may also account for this effect and is under investigation.

Controlled gene expression

H Bujard (Heidelberg University, Germany) presented recent developments of the now well-established, tetracycline-controlled expression system. In order to decrease immune responses to the HSV VP16 segment of the transactivator used, this protein domain was reduced to only 13 amino acids. The use of tissue-specific promoters upstream of the transactivator/repressor expression cassette allows the tissue-specific expression of genes in a doxycyline-dependent manner. The introduction of silencers decreased the influence of nearby enhancers close to null and made the system more sensitive. Combination of the two on and off systems allowed switching between the expression of two alleles, depending on the presence or absence of doxycycline. Whole arrays of mice, expressing either the transactivator expression cassette or the transgene of choice, are now available. NR Lemoine and colleagues (Imperial College School of Medicine, London, UK) are using tissue-specific promoters, such as the ERBB2 promoter, to limit expression of a suicide gene to tumor cells alone. This concept works well with plasmid DNA or retroviral vectors, with Ad insulator sequences required to reduce the influence of neighboring viral sequences on gene expression. Various promoter/suicide gene pairs are being examined and are entering clinical trials.

Infectious diseases

The use of gene therapy for the treatment of antibiotic-resistant bacterial infections reported by JN Norris (Medical University of South Carolina, Charleston, SC, USA). The bacteria are targeted with a modified lysogenic P1 phage in a suicide gene therapy approach. The group was able to package several plasmids encoding toxic agents, such as doc or chpBK, in the phage heads and to infect bacteria. Preliminary work on *Pseudomonas aeruginosa* is very promising. The ability to 'custom design' the phage tail opens the possibility for specific targeting of particular bacteria. KK Conzelmann (Gene Center of the Munich University, Germany) reported on the use of chimeric rhabdoviruses for targeting of HIV-infected cells. By constructing chimeric spikes, comprising the cytoplasmic tail of the spike glycoprotein G and utilizing cellular virus receptors, these authors were able to create a novel cell tropism. By using chimeric HIV receptors (CXCR4, CCR5 or CD4) instead of the G spike protein, the rhabdoviruses thus obtained were able to selectively target cells labeled at their surfaces with the HIV Env protein, offering a new and efficient method of delivery to these cells. DS Strayer (Jefferson Medical College, Philadelphia, PA, USA) reported on the use of recombinant SV40 viruses for gene delivery to human CD4+ cells in order to confer resistance to HIV infection. These vectors are devoid of the transforming T-antigen. Several therapeutic genes were delivered using this system without selection, leading to > 95% of the cells expressing the transgene. By using two rSV40, two therapeutic molecules were delivered to > 98% of the cells infected, thereby achieving a greater protection than with any single transgene alone.

Related fields

A highly interesting technique for the evolution and selection of enzymes by catalytic activity *in vitro* has been developed by D Tawfik and co-workers (Centre for Protein Engineering, MRC Centre, Sidney Sussex College, Cambridge, UK). In analogy to cellular compartmentalization, the required components for *in vitro* transcription and translation are enclosed in an artificial compartment, prepared by making water-in-oil emulsions of a defined

droplet size. The system is tested by selective enrichment of genes encoding enzymes that are able to convert a substrate that is linked to the gene. Using this technique in a model experiment, the *HaeIII* methylase gene was selectively enriched out of a 1×10^7-fold excess of genes encoding another enzyme. E Galun and co-workers (Hebrew University of Jerusalem, Israel) are using a highly sensitive CCD camera to detect light emitted from within animals. This way, they were able to detect luciferase reporter gene expression in the livers of Ad-transduced mice. This impressive technology currently costs about $90,000 and enables on-line monitoring of transgene expression in live animals.

Regulatory and industrial aspects

Parallel meetings of the Euregenethy group and of the European Society for Animal Cell Technology (ESACT) were of great importance, a sign of the increasing interest and action of regulatory affairs on gene therapy. K Cichutek (Paul Ehrlich Institute, Langen, Germany) described the differences between the regulation of the member states of the European Union and the need for a harmonization, especially for clinical trial regulations. M Papaluca-Amati (European Medicines Evaluation Agency, London, UK) announced the release of a draft document for gene therapy issues, addressing quality, preclinical and clinical aspects. This single, multidisciplinary document will be circulated for six months and comments and suggestions are more than welcome. O Cohen-Hagenauer (St Louis Hospital, Paris, France), who is coordinator of the Euregenethy group, reported on the problems encountered in the path to harmonization at the European level. The lack of a legal basis for harmonization might be amended soon, if a European Directive, currently actively discussed, is approved by the European Parliament. Euregenethy also called for the first Multidisciplinary Forum on Safety and Regulatory Issues in Gene Therapy to take place in June 2000 in Paris, France. The presence of the scientific community will be very important. M Wisher (BioReliance Ltd, Stirling, UK) presented data on the screening of producer cell lines for bacterial, viral and RCR (replication competent virus). Vigilance is required when developing cell lines for production of recombinant viruses for transfer into humans. Apart from the pathogens usually tested, the potential presence of bovine pathogens in medium and additives, including BSE agents, needs to be taken into account.

As far as ESACT is concerned, the seminars focused on the high-quality production of viral vectors for use in clinical protocols. E Morrey Atkinson (Targeted Genetics Corp, Seattle, WA, USA) presented a process for the production of adeno-associated virus (AAV) vectors utilizing a stable cell line in stirred-tank suspension bioreactors of > 100 l. The purification was then accomplished by industrial chromatography and filtration systems, leading to a sufficiently high purity to meet Good Manufacturing Practices (GMP) requirements. M-P Kieny (INSERM U74, Strasbourg, France), reported on the development of an immunogene therapy for the treatment of cervical cancer. This approach targets cells infected and transformed by papillomavirus, which in turn express the transforming proteins, E6 and E7. A phase I trial is underway with a recombinant, non-replicative vaccinia virus expressing these two proteins and an additional cytokine, IL-2. Three talks dealt with the optimization of industrial production of retroviral (OW Merten; Généthon III, Evry, France) and adenoviral vectors. Several parameters, including different cell lines, fixed bed or stirred-tank bioreactors, cells grown on microcarriers or in cell suspension, nutrient limitation and perfusion, were studied.